and progesterone was studied in rat and human uterus. The measurement of total (free and occupied) cytoplasmic and nuclear receptor was carried out by [^{3}H]-steroid exchange assays. Primary stimulation by estradiol in the rat uterus increased cytoplasmic and nuclear estradiol receptor (ERc and ERn) concentrations to about 10,400 sites/cell and 1260 sites/cell, respectively. However, administration of the above progestins brought about a dose dependent decline in the receptor concentration. The time course of changes in ERc indicated two phases of receptor replenishment, one between 3–9 h and second between 9–24 h. The second phase, which was partly dependent on protein synthesis, was sensitive to the inhibitory progestin block. Like ERc, progesterone receptor concentration (PRc) under the effect of progestins, decreased from an initial concentration of 8300 sites to 5100 sites/cell. Administration of norethindrone to women brought about a 50% decline in ERc and ERn levels. Similarly PRc levels in the proliferative phase of progestin treated women equalled those observed in mid secretory phase. Thus the modulation of uterine sensitivity to the hormones by limiting the receptor availability, appears to be one of the mechanisms by which progestins could exhibit the contraceptive effect at the uterine level.

96. Purification of sex hormone binding globulin by electrophoretic desorption from an affinity matrix

IQBAL, M. J. and JOHNSON, M. W., Department of Biochemical Endocrinology, Chelsea Hospital for Women, Dovehouse St., London SW3 6LT, England

A novel electrophoretic system for the purification of sex hormone binding globulin is described. The system utilises batch preparations of SHBG specifically immobilised on an affinity matrix (5α-androstane-3β,17β-diol-3β-hemisuccinate-Sepharose 4B) in a specialised small-scale electrophoretic cell. The electrophoretically desorbed protein was obtained in a purified and active form. The authors to date have achieved circa 1120 fold purifications using this single step procedure and succeeded in preparing 1.25 mg amounts of SHBG employing the cell in its present form. The application of the above principle to purification of a wide range of proteins using biospecific matrices together with results on the elution of glycoproteins from Concanavalin A-Sepharose, HSA from Cibacron Blue-Sepharose and steroid-specific antisera from steroid-Sepharose matrices are presented. The purified SHBG is characterised in terms of its molecular weight, electrophoretic mobility, amino-acid and carbohydrate composition.

6. MECHANISM OF ACTION

97. Oestradiol 2,4,6,7-[^{3}H] 17β uptake and subcellular distribution in the uterus of ovariectomized diabetic rat; induction of early protein synthesis

EKKA, E., DE HERTOGH, R. and VANDERHEYDEN, I., Endocrinology and Nutrition Unit, University of Louvain, UCL 5429, 1200 Brussels, Belgium

Whether the metabolic alterations in the diabetic subject has a bearing on the regulatory mechanisms of oestrogenic action at the cellular level is largely unknown. The present study was designed to examine some of these parameters in ovariectomized, streptozotocin induced diabetic rats. At the conclusion of a 4 h infusion with 17β-oestradiol 2,4,6,7-^{3}H ($E_2{}^3H$) plasma samples and uteri were analyzed for total, free and conjugated radioactivity (R.A.). The subcellular distribution of R.A. in the uterus was analyzed on sucrose density gradients and the effect of oestrogen on early protein (I.P.) synthesis was studied. The results show that the uterine uptake of $E_2{}^3H$ in the controls and diabetic rats was not significantly different. The plasma however, showed a significantly higher level of total R.A. in the diabetic rats, due to the higher concentration of conjugated moiety. In the uterus, the subcellular distribution of R.A. did not show any major difference between the two groups. Sucrose density gradients of cytosol and Kcl soluble nuclear extracts showed similar peaks in both groups. Finally, the stimulation of I.P. synthesis gave identical responses, showing that the I.P. synthesizing potential was not modified in the diabetics. In conclusion, streptozotocin induced diabetes of short duration (24 h–6 weeks), involving high glycemia but minor ketoacidosis did not modify the subcellular binding nor the hormonal activity of oestradiol in the rat uterus.

98. Physico-chemical characteristics of oestradiol and oestrone binding to macromolecules in the fetal uterus of guinea pig

NGUYEN, B. L. and PASQUALINI, J. R., C.N.R.S. Steroid Hormone Research Unit, Foundation for Hormone Research, 26 Boulevard Brune, 75014 Paris, France

Specific binding of oestradiol (E_2) and oestrone (E_1) were evaluated in fetal uteri throughout fetal development of the guinea pig. The values are similar for these two estrogens and increase with fetal development. After incubation of the cytosol fraction with 4.1×10^{-9} M [^{3}H]-E_2 or [^{3}H]-E_1, the specific binding of [^{3}H]-E_2 is (average of 5 experiments): 85 fmol/mg protein at 36–37 days of gestation, 390 at 44–45 days; 410 at 49–50 days; 720 at 60–66 days and 600 in newborns (3–4 days). For [^{3}H]-E_1 these values are, respectively, 74, 270, 350, 550 and 530. The K_D for [^{3}H]-E_2 is $2–4 \times 10^{-10}$ M and for [^{3}H]-E_1 $8–9 \times 10^{-10}$ M. Specific binding sites are also found in the nuclei after incubation of the total fetal uterine cell with [^{3}H]-E_1. Qualitative analysis of the radioactive material which was specifically bound to macromolecules shows that in the [^{3}H]-E_2 incubation 80–85% of the radioactivity remained as non metabolized E_2; similarly, for the incubation of [^{3}H]-E_1, 90–95% is non metabolized E_1. Oestrone competes with similar intensity for the formation of [^{3}H]-E_2 complexes and vice-versa, estradiol competes with the [^{3}H]-E_1 complex. It is concluded: (1) that specific uterine binding sites for E_2 and E_1 are present during intrauterine life, (2) that these specific binding sites increase during fetal development, (3) that the sites of binding are the same, and (4) that the conversion of oestrone ⇄ oestradiol is very limited in this fetal tissue.

99. Estrogen receptor: nuclear retention and uterotrophic activity of Centchroman; a comparison with estradiol-17β

DURANI, S., SAXENA, R., AGARWAL, A. K. and SETTY, B. S., Central Drug Research Institute, Lucknow, India

The temporal profile of estrogen receptor binding by the rat uterine nuclei (determined by exchange assay) and uterotrophic response following a single pharmacological dose of estradiol-17β (E_2), (2.5 or 10 μg/rat, s.c.) and Centchroman (C), (25 or 100 μg/rat, s.c.), a nonsteroidal estrogen possessing post-coital contraceptive activity, was examined. Both the doses of C caused prolonged elevation in the nuclear receptor (Rn) levels. A good correlation was found between the Rn levels and the temporal pattern of uterine response, the high dose giving a relatively more

prolonged increase in the Rn levels and the uterine weights (ut. wt.). The low dose of E_2 resulted in short term elevation in Rn levels followed by a steady decline reaching control values by 36 h; by 72 h the ut. wts. were also back to the control level. In the case of the high dose, however, an initial decline between 1 and 12 h was followed by a second increase in the Rn levels (peak value by 24 h) which declined to control level only by 36 h. This caused a more prolonged stimulation of the uterus: 72 h ut. wts. were 500% of the control. Although 100 μg of C and 10 μg of E_2 induced comparable Rn levels at 24 h (800 fmol/ut.), C was a weaker estrogen since in this case 72 h ut. wts. were only 250% that of the control.

100. Androsta-4,16-dien-3-ones, locally active anti-androgens

MARX, A. F., BOUWMAN, A. L., NYS, G. G. and JAITLY, K. D., Gist-Brocades N.V., Research and Development, P.O. Box 1, Delft, The Netherlands

Androsta-1,4,16-trien-3-one was the first compound synthesized in this series. It shows a strong anti-androgenic activity when applied locally in the chicken comb and the hamster flank organ tests. A draw-back was its penetrating musk-like odour, that many people found offensive. The anti-androgenic activity and the odour could be affected more or less independently of each other by the introduction of several substituents into the steroid nucleus. Finally we succeeded in synthesizing a compound which retained the strong local anti-androgenic activity and has an acceptable odour.

101. Ornithine decarboxylase in the chicken oviduct: evidence for involvement of post-transcriptional mechanisms in regulation by estradiol and progesterone

MESTER, J. and LEVY, C., Laboratory of Hormones, 94270 Bicêtre, France

When "withdrawn" chicken oviduct was incubated for 2 h at 37 C with estradiol (E_2) (20 nM) present in the culture medium containing amino-acids, the ornithine decarboxylase (ODC) activity increased 2–3 fold compared to the control incubated in the same medium without E_2, and ≃ 10 fold compared to non-incubated control. The E_2 concentration required for 1/2 maximum induction was ≃ 5 nM. The ODC induction was blocked by cycloheximide (25 μg/ml). Actinomycin D (10 μg/ml) did not interfere with the ODC induction by E_2 and had no effect on ODC activity in the absence of E_2; under these conditions the [^{3}H]-uridine incorporation into acid-insoluble fraction of the tissue was suppressed by ⩾85%. Alpha-amanitin (1 μg/ml) had no effect on ODC activity in the absence of E_2, and caused a "super-induction" of 25–30% when added together with E_2. Progesterone (P) at concentration ⩾10^{-7} M inhibited the ODC induction by E_2; the inhibition effect of P was not prevented when actinomycin D was included in the incubation medium. These results suggest that the regulation of ODC by E_2 and/or P may not require formation of new mRNA molecules.

102. Mode of entry of corticosterone into the liver cell

RAO, G. S., RAO, M. L., ECKEL, J., ALLERA, A. and BREUER, H., Institut für Klinische Biochemie der Universität Bonn, 5300 Bonn 1, Venusberg, Federal Republic of Germany

The first step in the action of steroid hormones is the permeation of the plasma membrane of the cell. We have investigated this critical process using corticosterone and the isolated rat liver cell. The uptake of corticosterone by the liver cell follows Michaelis–Menten kinetics. The hormone is taken up by at least two systems. A high affinity system with a K_t of 64 ± 40 nM and a low affinity system with a K_t of 1085 ± 313 nM. The physiologically important high affinity system is sensitive to NEM, FDNB and mercurithiosalicylate. Cortisone, cortisol, dexamethasone, aldosterone, testosterone, estradiol-17β and estrone reduce the uptake of corticosterone. Cell surface components such as carbohydrate and lipid are essential for uptake of the hormone. Intracellular cytosol binding proteins are not directly involved in the uptake process. Only the free, unbound hormone is taken up by the cell. The results indicate the presence of protein in the plasma membrane which act as carriers to transport the steroid into the cell.

103. Heterogeneity of the estrogen binding sites in human endometrium

KREITMANN, B., FAYE, J. C., MOURE, C. and BAYARD, F., INSERM U-168, C.H.U. Toulouse-Rangueil, 31054 Toulouse Cedex, France

The binding characteristics of oestradiol-17β (E_2), oestrone (E_1), oestriol (E_3) and 17α-ethinyloestradiol-17β (EE_2) in the cytosol fraction of human endometrium have been studied. It was observed that the estrogen binding sites are heterogenous: a similar number of binding sites (RE_2) are able to bind E_2 and EE_2 with high affinity ($5.9–7.7 \times 10^9\ M^{-1}$) and E_3 with a lower affinity ($1.6 \times 10^9\ M^{-1}$). A different number of binding sites (RE_1) are able to bind E_1 with high affinity ($5.3 \times 10^9\ M^{-1}$). The binding of labelled E_2 by RE_2 was not completely displaced by a 5000 fold molar excess of E_1 and two types of binding sites with different affinities for E_1 were apparent when the data were analyzed by the Dixon plot representation. Dilution of endometrial cytosol to variable extents had no effect on the ratio RE_1/RE_2. However electrofocusing on Sephadex G75 flat beds did not allow a separation of the specifically bound components (pH of 6.65 for RE_2 and 6.60 for RE_1, pH measured at 23 C). The proportion RE_1/RE_2 is variable during the menstrual cycle increasing during the luteal phase: there is a significant relationship between the ratio RE_1/RE_2 and plasma progesterone concentration.

104. Autoradiography of different fetal tissues of guinea pig, after *in vivo* and *in situ* injection of [^{3}H]-oestradiol

TARDY, J. and PASQUALINI, J. R., C.N.R.S. Steroid Hormone Research Unit, Foundation for Hormone Research, 26 Boulevard Brune, 75014 Paris, France

50 μCi of [^{3}H]-oestradiol (0.3 μg) dissolved in saline solution were injected subcutaneously and *in situ* to each fetus of Hartley Albino guinea pigs (60–66 days of gestation). Thirty min later the fetuses were removed and the different fetal tissues (uterus, kidney, lung and brain) were separated and frozen in isopentane, cooled by liquid nitrogen. Frozen sections (2–3 μm) were cut in a cryostat at −30 C and freeze dried with a cryopump. The freeze dried sections were dry-mounted on desiccated photographic emulsion (Kodak NTB-3)-coated slides which were exposed for 4–10 weeks at −18 C. The preparations were developed, fixed and then stained with methyl green pyronin. Significant quantities of radioactivity were localized in these fetal tissues mainly in the cell nuclei. In the fetal uterus, the radioactivity was principally concentrated in the endometrium and in the uterine glands, in the lung, in the alveolus, and in the kidney, in the inner zone of the medulla. A significant competitive effect was observed in the same tissues when the same quantity of [^{3}H]-oestradiol plus a 100 fold excess of unlabelled oestradiol was injected. These results agree with the data found in this laboratory on the presence of significant quantities of oestrogen receptors in these fetal tissues at the end of gestation.